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Original article

Protective effect of A/H1N1 vaccination in immune-mediated disease—a prospectively controlled vaccination study

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Abstract

Objectives. To assess the 2009 influenza vaccine A/H1N1 on antibody response, side effects and disease activity in patients with immune-mediated diseases.

Methods. Patients with RA, SpA, vasculitis (VAS) or CTD ($n = 149$) and healthy individuals ($n = 40$) received a single dose of adjuvanted A/H1N1 influenza vaccine. Sera were obtained before vaccination, and 3 weeks, 6 weeks and 6 months thereafter. A/H1N1 antibody titres were measured by haemagglutination inhibition (HAI) assay. Seroprotection was defined as specific antibody titre $\geqslant 1:40$, seroconversion as 4-fold increase in antibody titre.

Results. Titres increased significantly in patients and controls with a maximum at Week 3, declining to levels below protection at Month 6 ($P < 0.001$). Seroprotection was more frequently reached in SpA and CTD than in RA and VAS (80 and 82% and 57 and 47%, respectively). There was a significantly negative impact by MTX ($P < 0.001$), rituximab ($P = 0.0031$) and abatacept ($P = 0.045$). Other DMARDs, glucocorticoids and TNF blockers did not significantly suppress response ($P = 0.06$, 0.11 and 0.81, respectively). A linear decline in response was noted in patients with increasing age ($P < 0.001$). Disease reactivation possibly related to vaccination was suspected in 8/149 patients. No prolonged side effects or A/H1N1 infections were noted.

Conclusions. The results show that vaccination response is a function of disease type, intensity and character of medication and age. A single injection of adjuvanted influenza vaccine is sufficient to protect a high percentage of patients. Therefore, differential vaccination recommendations might in the future reduce costs and increase vaccination acceptance.

Key words: vaccination, rheumatoid arthritis, spondyloarthritis, H1N1, influenza.

CLINICAL SCIENCE

Introduction

Patients with immune-mediated diseases have been reported to be at increased risk for potentially lethal bacterial and viral infections, especially when treated with immune-suppressive medication [1, 2]. However, despite evidence, vaccination rate of this population is low [3, 4]. Traditional arguments against vaccination include reactivation of disease, insufficient response and vaccination side effects [5–7].

In 2009, discussion about vaccination was reactivated by the pandemic swine flu caused by an influenza variant A/H1N1. This encouraged us to study the immune response of immune-compromised patients and medical staff—both from our department—using a prospectively

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controlled vaccination protocol. As it was assumed that seasonal influenza and the variant A/H1N1 would not be covered by a single vaccine (either the seasonal or the A/H1N1 vaccine), all individuals were asked to get their annual vaccination against seasonal influenza. We then decided on a single injection of A/H1N1 vaccine in controls as well as in patients expecting an enhanced immune response due to the adjuvanted preparation.

Patients and methods

Between 11 November and 17 December 2009, a total of 189 participants were enrolled in the study comprising 149 patients and 40 healthy controls (Table 1). All individuals were seen, all data and sera collected and all vaccinations performed by one person (S.A.). Blood was collected prior to vaccination (T0), and 3 weeks (T1), 6 weeks (T2) and 6 months (T3) thereafter. Sera were kept at -20°C until analysis. Exclusion criteria were pregnancy, allergy to seasonal influenza vaccination or chicken eggs and former severe side effects after seasonal influenza vaccination. Participants received questionnaires regarding side effects (covering the first 5 days after vaccination and asking for fever, shivering, headache, bone pain, malaise, local pain at the site of injection, local redness, local swelling, ecchymosis, etc.). Diagnosis of influenza, hospitalization and/or urgent medical consultation had to be monitored separately. The study was approved by the local ethics committee [Kantonale Ethikkommission Bern (KEK)]. Patients gave written informed consent prior to study participation.

Vaccine

Participants received an i.m. single-shot of adjuvanted split influenza A/H1N1 vaccine [A/California/7/2009 (H1N1) v-like strain (X-179A) of 3.75 μg, containing AS 03 as adjuvant, Glaxo SmithKline]. Seasonal influenza vaccination with split inactivated influenza virus containing 15 μg each of the strains A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1) and B/Brisbane/60/2008 (Sanofi Pasteur MSD) had been performed in 127/149 patients and in 28/40 controls at a mean of 4 and 3.7 weeks, respectively, prior to participation. It was intended to allow for antibody production against seasonal influenza at first and to describe an effect of seasonal influenza vaccination to vaccination against A/H1N1.

Antibody assays

All sera were prepared for determination of antibody response at one time point and transferred at one time point by one person from Bern to Paris (J.W.). All determinations were performed in one effort in the Laboratory of Virology in Paris by one person (A.K.).

Table 1 Overview of patient characteristics

Patient characteristics	RA ($n=47$) *n* (%)	SpA ($n=59$) *n* (%)	Vasculitis ($n=15$) *n* (%)	CTD ($n=28$) *n* (%)	Controls ($n=40$) *n* (%)
Age, years					
<40	8 (17)	19 (32)	3 (20)	6 (21)	15 (38)
⩾40 to <60	15 (32)	32 (54)	6 (40)	14 (50)	22 (55)
⩾60	24 (51)	8 (14)	6 (40)	8 (29)	3 (8)
Gender					
Male	9 (19)	36 (61)	9 (60)	9 (32)	14 (35)
Female	38 (81)	23 (39)	6 (40)	19 (68)	26 (65)
Vaccination against seasonal influenza	43 (92)	46 (78)	14 (93)	24 (86)	28 (70)
Medication					
None	3 (6.4)	4 (6.8)	1 (6.7)	8 (29)	
Steroids					
<10 mg	17 (36)	5 (8.5)	10 (67)	4 (14)	
⩾10 mg	5 (11)	1 (1.7)	1 (6.7)	4 (14)	
DMARDs total	36 (77)	25 (42)	12 (80)	20 (71)	
SSZ/HCQ	4	3	0	7	
MTX	28	21	7	5	
Leflunomide	3	1	2	0	
AZA	0	0	2	4	
CSA	1	0	1	2	
Mycophenolate	0	0	0	2	
TNF-α	15 (32)	45 (76)	5 (33)	3 (11)	
TNF-α + MTX	10 (21)	16 (27)	4 (27)	3 (11)	
Other	21 (45)	6 (10)	4 (27)	5 (18)	
Rituximab	5	0	3	0	
Abatacept	10	6	0	4	
Tocilizumab	5	0	0	0	
CYC	1	0	1	1	

Antibody titres against A/H1N1 were tested by a haemagglutination inhibition (HAI) test modified from the Center for Disease Control (CDC) guidelines [8]. Briefly, sera were treated with receptor-destroying enzyme to remove non-specific inhibitors. Two-fold dilutions of treated sera, beginning 1 : 10, were tested against four haemagglutinin units of antigen [15 μg split inactivated influenza vaccine A/California/7/2009 (H1N1)-like strain (NYMC X-179 A)] vaccine as antigen for antibody measurement (in contrast to adjuvanted split influenza A/H1N1 vaccine [A/California/7/2009 (H1N1)v-like strain (X-179A) of 3.75 μg, containing AS 03 as adjuvant, Glaxo SmithKline] and 15 μg each of the strains A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1) and B/Brisbane/60/2008 (Sanofi Pasteur MSD) as regular vaccines as described in the vaccine section) (Sanofi Pasteur, Lyon, France) on human O Rh− red blood cells. The titre of HAI antibodies was defined as the highest serum dilution that completely inhibits haemagglutination. All sera of an individual patient were analysed on the same microtitre plate. Sera whose titres were <10 were assigned a titre of 5 for calculation purposes.

Seroprotection was defined as specific antibody titre ⩾1 : 40 (i.e. HAI), seroconversion as a 4-fold titre increase and the respective seroconversion rate. For interpretation of the data, we applied the European guidelines proposed by the Committee for Human Medicinal Products (CHMP) that are used for evaluation of influenza vaccines in a healthy population [9]. To our knowledge there are no comparable criteria in use when analysing vaccination in patients with immune-mediated diseases. Following these guidelines, one of the following criteria has to be fulfilled in order to assume sufficient protection in healthy subjects aged 18–60 years (>60 years): HAI ⩾1 : 40 in at least 70% (60%) of participants, seroconversion in at least 40% (30%) of participants and mean increase of the geometric mean titre (GMT) ⩾ 2.5 (2.0).

Statistical analysis

Data are described with statistical descriptive statistics. Multivariate regression analysis was performed by the generalized estimated equations model for titres and seroconversion; the multiple logistic regression model was applied for the seroprotection rate. A $P < 0.05$ was considered statistically significant. Confidence intervals were not calculated as they are not recommended for vaccination against influenza [10].

Results

Patients *vs* controls

Baseline seroprotection prior to vaccination measured by HAI ⩾1 : 40 was low in all groups. Protection rates and fulfilment of CHMP criteria are summarized in Table 2. While the three criteria are fulfilled in controls throughout the study period, seroprotection in patients declined from 70% at T1 to 29% at T3. Individual protection—i.e. protective values by either HAI or seroconversion—was reached in 39 (98%) controls and in 113 (75%) patients.

Vaccination response and type of disease

Response rates were better in SpA and CTD than in RA and vasculitis (VAS) (Table 3). The group of SpA patients showed immune protection throughout the study period. CTD patients showed the highest titre increase; however, none of the required criteria was met at T3. RA patients showed an unexpected low immune response, possibly in part explained by age, in part by medication (see below). VAS patients produced marginally sufficient values until T2.

Influence of medication

The strongest negative effects were seen with abatacept, rituximab and MTX ($P = 0.045$, $P = 0.031$ and $P < 0.001$). Patients treated with abatacept or rituximab never reached sufficient values regarding CHMP criteria. MTX

Table 2 Immune response in patients and controls

Patients versus controls	T1	T2	T3	T4	CHMP criteria
Patients ($n = 149$)					
HAI ⩾1 : 40, %	10	68	59	27	>70
GMT	8.5	47.7	36.2	19.6	
GMT ratio		5.6	4.3	2.3	>2.5
Seroconversion, %		64	54	33	>40
Controls ($n = 40$)					
HAI ⩾1 : 40, %	10	98	95	75	>70
GMT	8.7	116.0	93.0	51.0	
GMT ratio		13.3	10.7	5.9	>2.5
Seroconversion, %		85	80	65	>40

Table 3 Immune response separated for disease groups

Disease	T1	T2	T3	T4	CHMP criteria
RA ($n = 47$)					
HAI ⩾1 : 40, %	17	57	54	30	>70
GMT	9.7	40.8	33.3	20.9	
GMT ratio		4.2	3.4	2.2	>2.5
Seroconversion, %		53	43	28	>40
SpA ($n = 59$)					
HAI ⩾1 : 40, %	7	80	66	29	>70
GMT	7.1	61.3	42.0	19.5	
GMT ratio		8.6	5.9	2.7	>2.5
Seroconversion, %		75	63	42	>40
VAS ($n = 15$)					
HAI ⩾1 : 40, %	7	47	36	21	>70
GMT	9.5	26.4	24.2	14.9	
GMT ratio		2.8	2.6	1.6	>2.5
Seroconversion, %		47	40	20	>40
CTD ($n = 28$)					
HAI ⩾1 : 40, %	7	82	73	30	>70
GMT	9.1	51.6	38.9	20.0	
GMT ratio		5.7	4.3	2.2	>2.5
Seroconversion, %		68	61	29	>40

given subcutaneously at a mean dose of 15.8 mg/week in 61 patients (RA 16 mg, SpA 12.8 mg, VAS 15.4 mg, CTD 19 mg; range 7.5–30 mg) resulted in 15% lower titres than in the overall patient group at T1. Despite this reduction, protection was reached according to the GMT criteria until T2 (Table 4).

TNF blockers showed the least suppressive effect on antibody response and a 51% higher increase in titre compared with MTX-treated patients. The combination of MTX and TNF blocker inhibited immune response to a lesser degree than MTX alone ($P = 0.07$; Table 4).

Patients receiving DMARDs without MTX showed the second best response rates, resulting in immune protection throughout the study period ($P = 0.06$). Tocilizumab and cyclophosphamide both significantly impaired immune reaction leading to insufficient immune response. Glucocorticoids (GCs) at a mean dose of 7.4 mg/day did not significantly impair antibody response even when separating for doses <10 and $\geqslant 10$ mg/day ($P = 0.11$).

Patients without any immune-mediating medication ($n = 16$: RA 3, SpA 4, VAS 1, CTD 8) showed better responses compared with 133 patients with medication. Without medication there was a protection until T3, whereas this was reached only until T2 when taking any drug (data not shown). The increasing number of immune-mediating drugs taken had a negative impact on immune response (one, two or three drugs: $P = 0.01$; four drugs: $P < 0.001$).

Table 4 Immune response separated for medication

Medication	T1	T2	T3	T4	CHMP criteria
MTX ($n = 28$)					
HAI $\geqslant 1:40$, %	11	50	41	25	>70
GMT	8.7	32.5	26.1	18.6	
GMT ratio		3.8	3.0	2.2	>2.5
Seroconversion, %		50	36	29	>40
TNF-α ($n = 35$)					
HAI $\geqslant 1:40$, %	9	91	78	36	>70
GMT	7.9	83.3	57.8	22.4	
GMT ratio		10.5	7.3	2.8	>2.5
Seroconversion, %		83	66	46	>40
MTX + TNF-α ($n = 33$)					
HAI $\geqslant 1:40$, %	6	63	61	20	>70
GMT	6.9	37.6	28.3	14.3	
GMT ratio		5.4	4.1	2.1	>2.5
Seroconversion, %		64	61	27	>40
GCs ($n = 50$)					
HAI $\geqslant 1:40$, %	10.5	66.5	57	27.5	>70
GMT	10.6	55.2	38.7	21.8	
GMT ratio		5.2	3.7	2.1	>2.5
Seroconversion, %		59.5	43.5	26	>40
DMARDs ($n = 28$)					
HAI $\geqslant 1:40$, %	11	79	76	39	>70
GMT	9.5	73.4	55.4	26.9	
GMT ratio		7.7	5.8	2.8	>2.5
Seroconversion, %		75	64	46	>40
Abatacept ($n = 20$)					
HAI $\geqslant 1:40$, %	15	45	35	20	>70
GMT	9.3	23.8	24.2	15.8	
GMT ratio		2.5	2.6	1.7	>2.5
Seroconversion, %		35	30	10	>40
Rituximab ($n = 8$)					
HAI $\geqslant 1:40$, %	13	25	25	25	>70
GMT	10.0	21.0	22.9	16.2	
GMT ratio		2.1	2.3	1.6	>2.5
Seroconversion, %		25	25	13	>40

Influence of pre-vaccination, gender and age

Pre-vaccination with seasonal influenza vaccine and gender had no significant effect on antibody response to A/H1N1 vaccine ($P = 0.14$ and 0.76, respectively).

In contrast to controls, we found a linear decline in antibody response with increasing age in the patient group ($P < 0.001$). Patients <60 years of age showed immune protection throughout the study, in the large group $\geqslant 60$ years of age ($n = 52$) this lasted only until T2. The age effect was most pronounced in the SpA group with patients <40 years of age resembling responses in healthy individuals.

Side effects

Overall, there was no marked difference in side effects between the patient group and the control group.

Questionnaires were returned by 95 patients (64%) and 26 controls (65%), showing moderate symptoms of shivering (patients/controls, $n = 13/3$), headache ($n = 9/3$), joint pain ($n = 9/3$) and malaise ($n = 7/1$). Moderate-to-severe local pain was noted by 32 (34%) patients and mild local pain by 11 (42%) controls. There were no reports about rise in body temperature, influenza, hospitalization and/or urgent medical consultation.

Course of disease

An increase of disease activity was seen in 32 patients (15 RA, 12 SpA, 1 VAS, 4 CTD) during the entire study period. In eight patients, this was noted during the first 2 months after vaccination. The timely correlation might suggest a causal role of vaccination. Overall mild symptoms led to IA GC injections in one RA and one SpA patient and an increase of oral GCs in another RA patient.

Discussion

After influenza A /H1N1 vaccination, patients, analysed as one cohort, had a lower antibody response and a shorter duration of protective antibody levels than controls. Nevertheless, the CHMP criteria were fulfilled for the duration of an influenza season. Thus, despite a compromised immune system due to disease and/or immune-suppressive treatment, vaccination with a single dose of adjuvanted vaccine is sufficient to induce protection against influenza in patients with systemic autoimmune diseases.

The excellent vaccination responses seen in SpA and CTD patients are remarkable, as is the negative impact of MTX, abatacept and rituximab and, on the other hand, the minimal suppressive effect of TNF blockers.

The good responses in SpA patients might in part be due to their younger age (32 patients <40 years of age). This appears to be supported by the age-dependent response. However, interpretation is difficult as younger patients are over-represented in the SpA group and therefore influence calculations. Furthermore, age dependency was not found by other investigators [11]. In conclusion, patients with SpA are sufficiently protected by a single dose of adjuvanted vaccine, independent of age and medication.

In the RA group with most patients ⩾60 years of age (51%) we found the highest numbers of MTX, abatacept and rituximab used. Thus, age and medication combine in suppressing vaccination response. Data regarding medication-mediated effects on vaccination in RA are controversial. Some data report on lower, but still sufficient responses, without impairment by MTX [12, 13]. On the other hand, even a positive effect of MTX and a negative effect of TNF blockers on immune response to seasonal influenza vaccination were reported [14]. A likely explanation for the discrepant MTX results is dose and route of administration: in comparison with other centres we strictly administer MTX subcutaneously, and our mean dose of 15.8 mg/week is higher than that in most published cohorts. In lupus patients, a negative humoral as well as cell-mediated response to influenza vaccination is described, in particular during active disease, and possibly depending on the type of vaccine used [15]. In our group only, one of nine SLE patients had post-immunization antibody titres below recommended levels. This patient suffered active lupus nephritis and received cyclophosphamide until 2 months before vaccination followed by MMF plus low-dose GCs. A combination of disease activity and immunosuppression might have contributed to this negative response. The otherwise good response in the CTD group appears to be due to the higher number of patients without medication. As shown, medication and number of immune-mediating drugs negatively influence vaccination response.

Due to its B-cell-depleting properties, rituximab gained special attention regarding issues of vaccination. Several studies have led to the conclusion that vaccination, in particular vaccination against *Pneumococcus* species, should be performed prior to the first infusion [16, 17]. In our cohort, six of eight patients treated with rituximab showed insufficient antibody responses. Surprisingly, the two responders were vaccinated 1 and 3 months, respectively, after rituximab. None of the patients with insufficient response experienced infection so one might hypothesize that cellular immune response compensated for the humoral deficit [18]. The lack of a measurable immunosuppressive effect of GCs is probably best explained by the low mean dose and the lack of a dose dependency by the fact that most patients with low-dose GCs are treated concomitantly with DMARDs.

A positive effect of pre-vaccination against seasonal influenza was presumed and reflected in the high percentage of pre-vaccinated patients (85%) and controls (62%). Yet, pre-vaccination had no positive influence on the antibody response to the A/H1N1 vaccine in our study. This is in line with recent data from a healthy cohort including elderly subjects [19].

In contrast to a recent study on the influence of synthetic and biologic DMARDs on antibody response to the adjuvanted pandemic influenza vaccine, we used a single injection of vaccine instead of a repeated dose as well in controls as in patients leading to a sufficient antibody response in the majority of our patients without a single A/H1N1 infection [20]. We therefore still do not recommend a generalized second vaccine dose for patients receiving DMARDs of any kind. Furthermore, we measured vaccine response over a longer period of time. This allows for the information that patient's protection against pandemic influenza is no longer sufficient at 6 months after vaccination with regard to all three CHMP criteria. Yearly repetition of vaccination can hereby be supported.

The main weakness of this study is the heterogeneity and the small size of certain patient populations, the low number of controls over 60 years of age and low numbers of certain drugs. On the other hand, we were able to analyse the largest cohort of SpA patients published to date. Furthermore, the high number of patients treated with TNF blockers and/or MTX allowed robust statistical analysis and could lead to differential vaccination recommendations in the future.

The results of this study clearly show that vaccination response is a function of disease type, intensity and character of medication and of age. A single injection of adjuvanted influenza vaccine is sufficient to protect a high percentage of patients. Thus, recommendations of health authorities, which are largely based on results obtained with cohorts of healthy individuals, should not be extrapolated to patients with immune-mediated diseases.

Rheumatology key messages

- Pandemic influenza vaccination is effective and safe in patients with immune-mediated diseases.
- A single-dose adjuvanted A/H1N1 vaccine protects the majority of patients with immune-mediated diseases.
- Yearly influenza vaccination is recommended because of the loss of viral protection 6 months after vaccination.

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